




## COMMENTARY

# A breakthrough in understanding the molecular basis of coral heat tolerance

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Reef-building corals are cnidarian animals (Anthozoa, Scleractinia) that mostly live in colonies composed of hundreds of thousands of tiny coral polyps. The polyps house dinoflagellate algal photosymbionts of the family Symbiodiniaceae inside their gastrodermal cells (Fig. 1A), and this mutualistic association builds the three-dimensional structure of the reefs via deposition of calcium carbonate skeletons. Scleractinian corals thus support a tremendous diversity and biomass of coral reef organisms. However, much of the biology of corals is poorly understood, partly due to a lack of advanced genetic tools for both the corals and the Symbiodiniaceae. In PNAS, Cleves et al. (1) catapult the field forward by describing an efficient protocol for applying the CRISPR technology to the coral *Acropora millepora*. They show that a CRISPR knockout of the gene encoding Heat Shock Transcription Factor 1 (*HSF1*) in larvae of this coral reduces their heat shock tolerance, thus confirming a role for *HSF1* in protecting corals from heat stress.

Gaining a deeper understanding of the coral heat stress response is urgent given that climate warming, and particularly the resulting increased frequency and severity of summer heat waves, are responsible for the alarmingly rapid loss of coral reefs worldwide. Exposure to elevated temperature can result in the loss of the symbiotic algae from the coral tissues, a process known as coral bleaching (Fig. 1B). The algae normally translocate much of their photosynthetically fixed carbon to their coral host, thus meeting most of the nutritional needs of the coral. When corals are bleached and unable to repopulate their algal symbionts during prolonged periods of excessive heat, however, they starve and eventually die. For example, approximately one-half of the corals on Australia's Great Barrier Reef died due to the two back-to-back mass bleaching events of 2016 and 2017 (2).

A suite of non-genetically-modified (GM) bioengineering approaches is currently being explored for their ability to augment the thermal bleaching tolerance

of corals (3, 4). These include the following: 1) transplantation, or assisted gene flow, i.e., the translocation of coral colonies or spawning slicks (embryos and larvae of a mix of broadcast spawning species) to other localities; 2) hybridization or gene pool mixing, i.e., the ex situ crossing between colonies from distinct species or populations; 3) selective breeding, i.e., the ex situ crossing between conspecific colonies from the same population based on their trait values (e.g., high thermal tolerance); 4) preconditioning, i.e., the deliberate exposure of corals to sublethal stress conditions to induce additional tolerance to subsequent stress events; and 5) microbiome manipulation, i.e., the inoculation of corals with wild-type or experimentally evolved Symbiodiniaceae, bacteria or other microbes that confer enhanced stress tolerance to the coral.

Small- or laboratory-scale success has been achieved for several of these approaches [e.g., transplantation (5), hybridization (6–8), Symbiodiniaceae experimental evolution (9), and bacterial probiotics (10)]. However, several of these approaches would benefit greatly from knowing the biochemical processes that contribute to thermal tolerance, the genes whose expression can be altered to enhance tolerance, and the changes in their expression required to achieve that. That knowledge could guide the design, selection of starting materials, and methods for transplantation, hybridization, and selective breeding experiments.

Elevated temperatures are hypothesized to trigger bleaching via an overproduction of reactive oxygen species by the algae, which in turn could be caused by either damage to their photosystems or a breakdown of the nutritional exchange between the algae and their hosts (11). There is widespread and compelling evidence that genetic differences in the algae contribute significantly to individual differences in thermal bleaching tolerance within coral species (5, 9, 12, 13), but genotypic effects in the host also explain a

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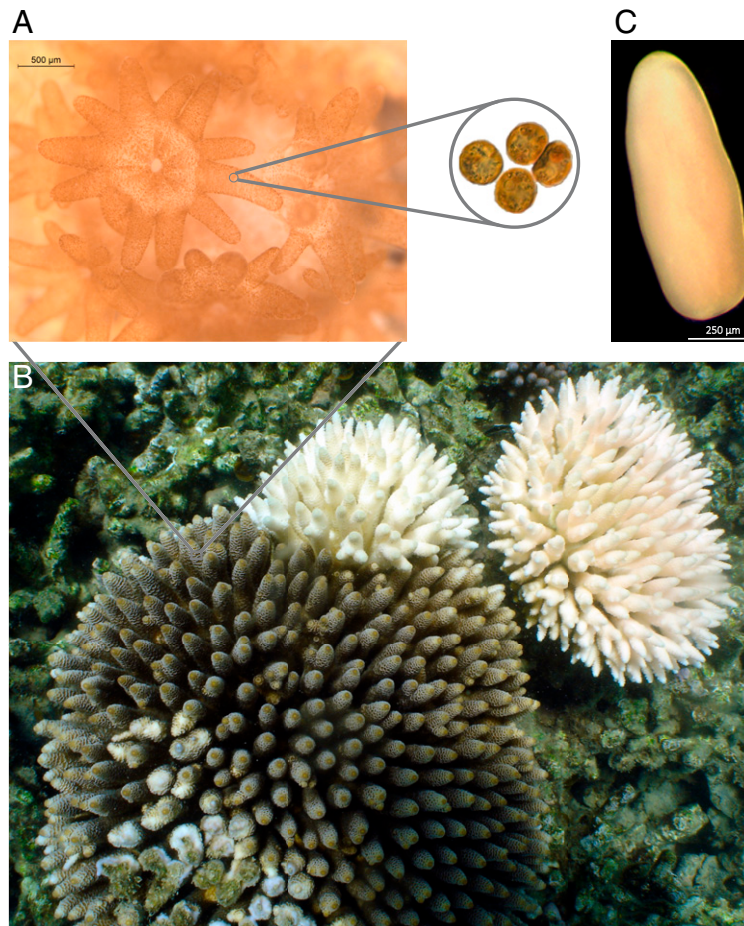
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**Fig. 1.** The scleractinian coral, *Acropora millepora*. (A) Close-up of individual polyps showing the endosymbiotic Symbiodiniaceae algae as brown pigmentation and as individual cells in the Inset. Symbiodiniaceae cells are ~5 to 10 μm in diameter. Image credit: Katarina Damjanovic (photographer). (B) Bleached and unbleached adult colonies during a natural bleaching event on the Great Barrier Reef. Image credit: Ray Berkelmans (photographer). (C) Aposymbiotic larva. Image credit: Kate Quigley (photographer).

considerable portion of the variance (14), and the heritability of thermal tolerance in aposymbiotic larvae (i.e., those lacking algal symbionts; Fig. 1C) is high (7). The CRISPR technique developed by Cleves et al. (1) currently works as a knockout capability, and their paper provides an impressive demonstration of its utility by showing that ablation of *HSF1* function reduces the heat tolerance of aposymbiotic larvae.

However, as the authors note, what is ultimately needed is to enhance tolerance, which may require overexpression of key genes (9). It will likely require a knockin technology to assess whether such overexpression does indeed enhance thermal tolerance. While a knockin technology has not been developed as yet, the CRISPR technique developed by Cleves et al. (1) is a crucial foundation for building such a capability.

Whether overexpression of *HSF1* itself would enhance thermal tolerance is unclear. In higher organisms at least, much of the regulation of its expression occurs posttranslationally (15). Additionally, while *HSF1* does enhance the expression of *HSP70* and *HSP90* heat shock genes in some organisms, and elevated expression of *HSP70* and some other *HSPs* is known to confer higher thermal tolerance (16), it also affects the expression of several other genes involved in other physiological processes whose disruption could have adverse pleiotropic effects on the organism. Finally, overexpression of *HSP70* can in turn repress

*HSF1* expression (17). Notably, Meyer et al. (18) found *HSF1* expression was down-regulated in aposymbiotic *A. millepora* larvae exposed to elevated temperature.

There are in fact many other candidate genes whose overexpression might boost coral thermal tolerance. Genome-wide single-nucleotide polymorphism (SNP) analyses suggest that thermal tolerance is a polygenic trait in corals (19, 20) and transcriptome studies on corals exposed to elevated temperature provide useful lists of specific genes with roles in the unfolded protein response, immune functions, and oxidative stress responses in other organisms, which may contribute to coral thermal tolerance (21). Two cautions here are that the SNP analyses suggest there may be few genes that individually exert a major effect, and the transcriptome studies suggest different candidate genes depending on the conditions of the heat stress and the species under investigation. However, the same situation applies in other organisms like *Drosophila* species, and in those cases several individual SNPs have still been shown to exert measurable effects on tolerance (22).

Another important extension of Cleves et al. (1) will be to test whether knockouts (and in due course knockins) of genes affecting the heat tolerance of aposymbiotic coral larvae translate to parallel effects on the thermal bleaching tolerance of mature corals containing their algal symbionts. This should be

readily testable given the ability to inoculate aposymbiotic larvae with symbionts in the laboratory, settle them, and rear them through to adulthood.

The CRISPR technology described by Cleves et al. (1) is also not limited in its utility to heat and bleaching tolerances. It will be just as useful in interrogating the molecular bases of other traits important to the ongoing health of coral reefs, such as tolerance of acidification and various pollutants and resistance to a variety of pathogens. Intriguingly, the coral genomes sequenced to date have a high proportion of unigenes, i.e., genes for which there is insufficient similarity to those in other species to suggest their biological function (23). There is thus the enticing prospect that application of CRISPR technology to corals may expose entirely novel molecular mechanisms for some of these stress responses and tolerances.

Cleves et al. (1) conclude by considering the possibility that their CRISPR technology could be used not just to guide other approaches to the development of more tolerant coral strains but to generate such strains directly. Strains generated in this way would be classified as GM in some jurisdictions, and in any event their release into the environment would, very properly, face rigorous regulatory and public scrutiny. While acknowledging that increasingly sophisticated and precise gene-editing technologies are being developed that could reduce potential risks (24), we concur with the authors that the focus should remain on non-GM approaches and strains in the near to midterm future.

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